FEBS Letters 585 (2011) 967–972

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Re-evaluating the general(ized) roles of AMPK in cellular metabolism

Julie Mantovani, Richard Roy *

Department of Biology-DBRI, 1205 avenue Docteur Penfield, McGill University, Montreal (QC), Canada H3A 1B1

ARTICLE INFO

Article history:
Received 19 November 2010
Revised 13 December 2010
Accepted 13 December 2010
Available online 21 December 2010

Edited by Wilhelm Just

Keywords:
AMP-activated protein kinase
LKB1
Metabolism
Lipogenesis
Metformin
ATP
Mitochondria

ABSTRACT

AMPK is a protein kinase activated by various cellular stresses such as glucose deprivation, hypoxia or exercise. Despite having been studied for decades only a limited number of targets have been well described in tissues as varied as liver, muscle, and adipose tissue. Recent studies have shown that AMPK does not function in a similar manner, or through identical targets, in all cellular situations, posing challenges to some accepted paradigms describing AMPK function. A combination of genetic models and cell biological analysis of AMPK function in specific cell/developmental/environmental contexts will be required to accurately complement our understanding of the role(s) of AMPK in cancer, diabetes and other diseases.

The effects of glucose deprivation, hypoxia and numerous other cellular stresses trigger the activation of the AMP-activated protein kinase (AMPK), prompting it to appropriately re-distribute resources to provide the cell with the required energy for survival. To do this, AMPK will alter cellular metabolism by blocking ATP-consuming pathways, while activating those that generate ATP. These include a marked increase in glucose uptake, a stimulation of fatty acid oxidation, and an inhibition of glycogen, fatty acid, cholesterol, and protein synthesis. The action of AMPK can be acute due to a direct phosphorylation of target proteins, such as various metabolic enzymes, or it can be more long-term through its ability to phosphorylate critical transcription factors that regulate the expression of metabolically-responsive target genes. Although AMPK has long been described as a master metabolic regulator, the identification of the key kinase substrates required to adjust its characteristic metabolic outputs downstream of cellular energy stress has been limited to ~20 targets, most of which cannot solely account for the major cellular changes that occur upon activation of this stress pathway. Furthermore, the spectrum of targets and the effects of AMPK activation downstream of nutrient stress appear to vary between cell types and developmental contexts, making it increasingly difficult to make large general conclusions about the various AMPK-dependent outputs. We highlight a few of these situations here, emphasizing the importance of re-evaluating many of the current paradigms dictating our understanding of how AMPK may affect the specific cellular changes that occur in response to reduced cellular energy levels.

These events occur in most tissues, but are best understood in the liver, muscle, heart and adipose tissue where AMPK may have cell/tissue/developmental context-specific consequences. This is perhaps best reflected in the regulation of lipid metabolism downstream of AMPK activation. In many organisms, the response to reduced cellular energy stores impinges on AMPK which will attenuate lipogenesis in organs such as muscle and liver, in part through the phosphorylation of two well-characterized targets: Acetyl-CoA Carboxylases 1 and 2 (ACC1 and ACC2). These enzymes catalyze the synthesis of malonyl-CoA, a pivotal substrate for fatty acid synthesis and the regulator of fatty acid oxidation. The functional distinction between ACC1 and ACC2 lies in their subcellular distribution; ACC1 is cytoplasmic whereas ACC2 is associated with mitochondria. The inhibitory effects on fatty acid synthesis downstream of AMPK activation are likely mediated by the AMPK-dependent phosphorylation and subsequent inhibition of ACC1. On the other hand, the AMPK-mediated stimulation of fatty acid oxidation is achieved by direct phosphorylation and inhibition of ACC2 [1]. Both of these events result in a general reduction of lipid stores and consequent ATP production in response to energy stress.

Although this scenario is well accepted and likely highly conserved among many organisms, AMPK does not always affect lipid metabolism in this generalized manner. In the nematode *Caenorhabditis elegans*, larvae subjected to inadequate growing conditions opt to execute an alternative developmental stage referred to as dauer, where animals stop foraging and prepare, both

* Corresponding author. Fax: +1 514 398 5069.
E-mail address: richard.roy@mcgill.ca (R. Roy).

0014-5793/$36.00
doi:10.1016/j.febslet.2010.12.015

morphologically and metabolically, to survive environmental extremes. This developmental switch is controlled in part by insulin signaling and at least two other highly conserved signaling pathways and is associated with major changes in metabolism, cell division and behaviour.

Upon executing the dauer program, larvae accumulate lipid as a long-term energy resource, while implementing a general state of developmental quiescence. Some of these changes are AMPK-dependent, and in its absence many of these controls go awry. Particularly noteworthy, the germline stem cells of the AMPK mutant dauer larvae exit quiescence and begin to hyperproliferate giving rise to a marked hyperplasia [2]. In addition, the increased lipid stores typical of the dauer larvae become rapidly exhausted, which eventually leads to premature lethality in these mutant dauer animals. Although both events occur through independent downstream effectors, both are under the control of the upstream regulators of AMPK, namely LKB1 and STRADα. The literature is rich in references that link AMPK activity to hormone sensitive lipase (HSL), particularly in muscle cells during intense bouts of exercise-associated energy stress [3]. It would therefore seem intuitive that a similar interaction between HSL and AMPK would be responsible for the observed response in lipid hydrolysis at the onset of dauer. Curiously, in AMPK mutant dauer larvae, the loss of HSL has no effect on the accelerated lipid hydrolysis. Alternatively, AMPK was demonstrated to act through the phospho-inhibition of a triglyceride lipase called ATGL-1 to block lipid hydrolysis during this developmentally quiescent stage [4]. In wild type dauer larvae the AMPK-dependent inhibition of ATGL-1 activity allows the lipid stores to accumulate and persist, presumably to provide a threshold level of energy over the course of the diapause for the nutrient-challenged dauer larva. It is not clear how AMPK affects other enzymes normally involved in lipogenesis during this transition (Fig. 1). However, the accumulation of lipid in AMPK mutant dauers seems quite similar to wild type animals suggesting that lipogenesis is not adversely affected by its absence. The parallels of dauer with other organisms are quite compelling and similar mechanisms may be at play in animals that undergo extended periods of inactivity or hibernation.

One of the major metabolic pathways that drive cellular ATP production is glycolysis and, not surprisingly, AMPK activity can influence glycolytic flux, although its function in this regard remains controversial. In skeletal muscle, one of the principal roles of AMPK is to stimulate glucose uptake via the translocation of the glucose transporter GLUT4 to the plasma membrane. Phosphorylation of the Rab-GAP protein TBC1D4 is regulated by AMPK

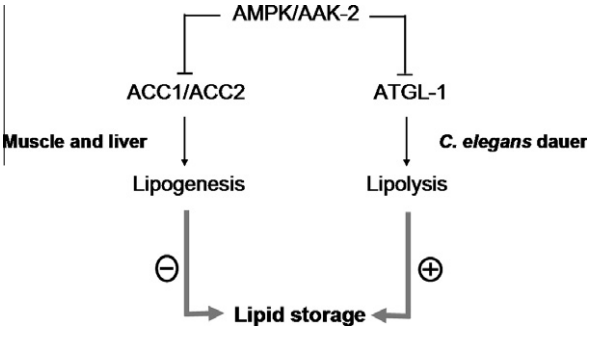

Fig. 1. Opposite effects of AMPK on lipid storage and hydrolysis. The regulation of lipid turnover that occurs following the activation of AMPK/AAK-2 cannot be generalized and is highly dependent on both the cellular and developmental context. In mammalian cells in culture, activated AMPK blocks lipogenesis by phosphorylating key mitochondrial enzymes involved in lipogenesis. In contrast, during the *C. elegans* dauer stage, the activity of a critical triglyceride lipase (ATGL-1) is phospho-inhibited by AAK-2, one of the two catalytic subunits of AMPK in *C. elegans*, allowing the larva to utilize the stored lipids as a long-term energy resource.

in skeletal muscle [5], while the closely related paralog TBC1D1 has also been shown to be phosphorylated by AMPK in response to insulin and contraction [6,7]. The GLUT1 transporter can also be activated by AMPK, although the critical kinase target involved remains yet to be identified [8]. AMPK has been shown to stimulate glycolysis in heart through phosphorylation of PFK2 at Ser-466 [9]. The biguanide metformin, which acts as an indirect activator of AMPK, can also stimulate glycolysis in cells in an AMPK-dependent manner [10]. However, AMPK activity does not always correlate with increased glycolysis. AICAR, an AMPK agonist, can actually inhibit glycolysis in multiple cell types in an AMPK-dependent fashion [11–13]. It is possible that the effect of AMPK on glycolysis may depend on either tissue type or the context in which AMPK is activated. Future work using tissue-specific deletions of AMPK should help to clarify the controversy surrounding the role of AMPK in this critical process.

Many of the characteristic AMPK-dependent changes that occur in muscle are not due to direct phosphorylation of enzymatic substrates, but rather due to chronic changes in the muscle-specific transcriptional repertoire downstream of AMPK activation. This is achieved through the phosphorylation of different transcription factors expressed in the muscle, such as the transcriptional co-activator Peroxisome proliferator-activated receptor Gamma Coactivator 1-alpha (PGC-1α), the transcription factor GLUT4 Enhancer Factor (GEF), and/or the derepression of the transcription factor Myocyte Enhancer Factor-2 (MEF2) via phosphorylation of Histone Deacetylase 5 (HDAC5) [14]. Each of these transcriptional changes will favor GLUT4 transcription and its subsequent expression and translocation to its site of function at the plasma membrane. These effects occur quite rapidly after a bout of exercise resulting in a concomitant increase in the rate of glucose uptake. Thus, AMPK will control multiple aspects of myocyte homeostasis following exercise and muscle contraction to allow the skeletal muscle to adapt to this specific physiologic state.

In the liver, one of best-documented effects of AMPK is its suppression of hepatic gluconeogenesis through the action of a number of transcription factors that include CREB Binding Protein (CBP) [15], its co-activator CRTC2 [16], and the orphan nuclear receptor SHP [17]. SHP has been shown to be an important contributor of AMPK-dependent suppression of CREB/CRTC2-mediated hepatic gluconeogenic gene expression [18]. These modifications will, *inter alia*, down-regulate the expression of gluconeogenic genes such as Phosphoenolpyruvate Carboxykinase (PEPCK) and Glucose-6-Phosphatase (G6Pase) [19]. These enzymes regulate two important reactions of gluconeogenesis: the synthesis of PEP from oxaloacetate and that of glucose from glucose-6-phosphate (G6P). Therefore, by blocking the activity of these critical transcription factors, AMPK can decrease the rate of gluconeogenic flux.

The typical acute/chronic duality of the AMPK response is yet further reflected in its ability to reduce the expression of genes that regulate lipogenesis. In addition to its acute effects on ACC1/2, AMPK phosphorylates and thereby inhibits the Carbohydrate-Responsive Element-Binding Protein (ChREBP) by decreasing its DNA binding activity [20], whereby it affects the expression of ACC1 and Fatty acid Synthase [21]. Moreover, activation of AMPK also inhibits the Sterol Regulatory Element Binding Protein-1 (SREBP1-c) [22], which is required for the appropriate expression of ACC1 [23].

Therefore, in response to metabolic imbalance, AMPK initiates both short term and long-term effects to right bioenergetic homeostasis. The "first wave" response by AMPK is mediated through post-translational modifications which can acutely affect the activity of key metabolic enzymes/effectors (i.e. eEF2, PFK2 or mTORC1), while in the long term, activated AMPK can alter gene output by phosphorylating transcription factors, transcriptional cofactors, chromatin modifying enzymes including histone deacetylases

[24], or through the direct modification of histones to alter chromatin configuration [25], thereby modulating target gene expression in response to cellular energy stress.

Sirtuins are highly conserved NAD^+-dependent histone/protein deacetylases. These enzymes have been shown to regulate diverse cellular processes that range from inflammation, metabolism, cellular senescence/aging to cellular apoptosis/proliferation, differentiation, stem cell pluripotency, and cell proliferation [26]. The best-known and most studied member of this family is the Silent Information Regulator T1 (SIRT1). SIRT1 is able to deacetylate lysine residues in a reaction that consumes NAD^+, and releases nicotinamide, *O*-acetyl ADP ribose (AADPR), and the deacetylated product [26]. For this reason, the activity of SIRT1 is highly dependent on the intracellular levels of NAD^+. The generation of NAD^+ in the cell can occur de novo via the use of tryptophan, or alternatively through the more energy efficient Salvage Pathway. The enzymes required for NAD^+ salvage include NAMPT (nicotinamide phosphoribosyltransferase) and NMNAT (nicotinamide mononucleotide adenylyltransferase) and are thus critical for the regulation of SIRT1. SIRT1 responds to numerous cellular stresses such as fasting, exercise, or low glucose availability, all of which are also associated with increases in the cellular levels of NAD^+ [28]. AMPK activity has been shown to increase cellular NAD^+ levels [28], although whether this occurs through regulation of the NAD^+ salvage pathway remains unclear The characteristic lifespan extension that occurs in yeast, *Drosophila melanogaster* and *C. elegans* following caloric restriction or conditions of nutrient stress are also associated with SIRT1 activity [27,29] and it is likely that SIRT1 can regulate these processes through its ability to deacetylate histone or non-histone proteins [30]. SIRT1 and AMPK have many common actions on various processes such as metabolism and mitochondrial function and recently, the link between these two sensor proteins has become more obvious.

Using heterozygous SIRT mice under conditions of glucose restriction (GR), it was shown that AMPK, NAMPT and SIRT1 are part of the same pathway that allows skeletal muscle cells to sense and react to nutrient availability [31]. GR activates AMPK which induces NAMPT transcription in a SIRT1 dependent manner, consequently augmenting the cellular NAD^+:NADH ratio.

The mechanism through which AMPK enhances SIRT1 activity appears to rely on increases in cellular NAD^+ levels, although this seems to be at odds with the function of AMPK in fatty acid oxidation, which should result in increased cellular levels of NADH. How the cell perceives these changes and executes the appropriate downstream events remains yet to be elucidated. Nevertheless, the NAD^+-dependent activation of SIRT1 is responsible for the deacetylation of targets that include PGC-1α, FOXO1 and FOXO3a [28]. Similarly, AMPK is necessary to act as the primary sensor in C2C12 myotubes during fasting and exercise to activate SIRT1 and its downstream targets [32]. Therefore, taken altogether these recent data indicate that both AMPK and SIRT1 cooperate in a common pathway through their independent roles in regulating lipid metabolism and mitochondrial biogenesis and that many of the observed metabolic effects of SIRT1 may be, in fact, responses to cellular changes mediated by AMPK function.

Although the recent link made between AMPK and SIRT1 clarifies a number of related metabolic phenomena associated with each of these gene products, it has been well established that AMPK activation exerts a substantial impact on many of the regulatory components involved in mitochondrial biogenesis and/or function.

Long-term exercise training increases oxidative capacity in muscle through its positive effects on mitochondrial biogenesis resulting mainly from the metabolic and nutrient stress that occurs within cells. Various studies have shown that AMPK is required for the increased numbers of mitochondria observed in skeletal muscle in response to energy deprivation in [33,34]. However, the understanding of the AMPK-dependent mechanism that regulates these mitochondrial processes has been significantly enhanced through identification and characterization of key AMPK targets involved in mitochondrial biogenesis. The over-expression of a mutant variant of the AMPKγ3 subunit increases mitochondrial biogenesis in glycolytic skeletal muscle. This is correlated with a significant increase in the expression of PGC-1α and several transcription factors that regulate mitochondrial biogenesis including Nuclear Respiratory Factor 1 (NRF-1), NRF-2 and Transcription Factor A, Mitochondrial (TFAM) [35].

PGC-1α is a master regulator of oxidative metabolism and is able to cooperate with a number of transcription factors that control metabolic and mitochondrial gene expression, including the Peroxisome Proliferator-Activated Receptors (PPARs), the thyroid hormone receptor, glucocorticoid receptor, estrogen receptor, MEF-2 and FOXO transcription factors [36]. In general, through its coactivator function, PGC-1α will increase mitochondrial activity during conditions of energy deficiency, and alternatively attenuate it when the energy requirement is low. Moreover, in addition to its effects on NAD^+-dependent SIRT1 activation, AMPK can also activate PGC-1α through a direct interaction and resulting phosphorylation in skeletal muscle [37]. Not surprisingly the transcriptional expression profile that is affected by PGC-1α corresponds quite closely to the spectrum of gene activities that also fall under AMPK control, suggesting that PGC-1α is the major effector regulating mitochondrial biogenesis downstream of AMPK activation.

Recently, a number of drugs that modify mitochondrial function have been developed for use in the clinic (Table 1). Metformin is the widespread drug of choice for treating type II diabetes worldwide and recent estimates predict that 42 million patients are being prescribed the drug in the USA alone. Its efficiency in lowering blood glucose and controlling insulin levels in diabetic patients is unquestionable, although until recently its mechanism of action has eluded investigators.

Metformin is thought to be a weak mitochondrial poison that targets complex one of the electron transport chain (ETC) in mitochondria. The ETC is the major site of oxidative phosphorylation where it uses the energy released through the oxidation of nutrients to produce ATP. The ETC is composed of 5 complexes that convert the energy released following the transfer of electrons to produce a proton gradient across the mitochondrial inner membrane. This electrochemical proton gradient is then used by ATP synthase to generate ATP from ADP and Pi. It is through this effect that metformin is capable of modifying the cellular energy charge, which was assumed to be countered by the induction of AMPK and thereby mediate the beneficial effects of the drug [22].

However, several recent studies have challenged this idea. It has proven quite difficult to demonstrate any significant increase of the AMP:ATP ratio in isolated cells (muscle, Chinese hamster ovary fibroblasts or the rat hepatoma cell line H4IIE) following treatment with metformin [38,39]. However, this may be a technical hurdle that reflects the ability (or inability) to accurately measure active or free adenine nucleotide levels as opposed to total; which comprises mostly protein-bound/sequestered nucleotides, in various cell types, since in studies conducted with intact hepatocytes, the ADP:ATP ratio in both the cytosolic and the mitochondrial compartments increased after metformin administration [40]. Similarly, metformin-induced increases in the AMP:ATP ratio have been recently shown in primary hepatocytes and the liver of mice [41], while after a perfusion of metformin in intact rat heart, an increase of AMPK activity correlated with a increase in cytoplasmic AMP [42]. It is noteworthy that in this study both the total AMP content and the total AMP:ATP ratio did not change. Therefore, it is difficult to conclude whether indeed metformin alters the cellular AMP:ATP ratio as it may depend on the cellular context or, more likely, the sensitivity of the quantitative methods employed.

Table 1
Drugs used to detect modification of AMP:ATP and/or ADP:ATP ratio in different contexts. For each drug, the model and the maximal concentration used is indicated, together with the variation observed for the ratios.

Drug		Ratio AMP:ATP	Ratio ADP:ATP	Model	Maximal concentrations used	References
Biguanides	Metformin	=		H-2K^b cells (skeletal muscle, mouse)	2 mM	[36,40]
				Heart (rat)	10 mM	
			=	CHO and H4IIE cells (hepatoma, rat)	5 mM	[37,41]
				HEK 293 cells (kidney, human)	3 mM	
		↑		Primary hepatocytes (mice)	2 mM	[39]
				Liver (mice)	20 mg/kg	
			↑	Liver (rats)	2 mM	[50]
	Phenformin	↑		Brain slices (rat)	5 mM	[51]
			↑	HEK 293 cells	3 mM	[41]
	Galegine		↑	HEK 293 cells	0.1 mM	[41]
A-769662		=		Primary hepatocytes (rat)	0.1 mM	[41,52,53]
				HEK 293 cells	0.3 mM	
PT1		=		L6 myotubes (rat)	0.08 mM	[54]
Thiazolidinediones	Rosiglitazone	↑		H-2K^b cells	0.2 mM	[36]
	Troglitazone		↑	HEK 293 cells	0.1 mM	[41]
	Pioglitazone	↑		Liver (rat)	3 mg/kg	[55]
Barbiturate	Phenobarbital		↑	HEK 293 cells	3 mM	[41]
Phytochemicals	Berberine		↑	HEK 293 cells	0.1 mM	[41]
		↑	↑	L6 myotubes	0.02 mM	[56]
	Quercetin		↑	HEK 293 cells	0.3 mM	[41]
	Resveratrol		↑	HEK 293 cells	0.3 mM	[41]
AICAR			↑	Hepatocytes (rat)	0.1 mM	[57]
			=	HEK 293 cells	3 mM	[41]

The recent generation of cells that express an AMP-insensitive AMPK variant through the overexpression of mutant forms of the AMPKγ subunit has provided additional clues that have helped to discriminate between these different observations [43]. A plethora of compounds were used to test the response of wild type or mutant expressing cells and, although many of these compounds affect the ADP:ATP ratio significantly, metformin did not. The authors do indicate that metformin was indeed capable of activating AMPK, presumably by increasing cellular AMP concentrations. Again, this may stem from the inability to accurately quantify very low AMP levels as this ratio varies as the square of the ADP:ATP ratio [44]. It seems that the change in the AMP:ATP ratio, or perhaps more accurately, the lack thereof, cannot be used as an accurate measure of AMPK activation. The degree of change and/or the critical threshold of AMP that might cause AMPK activation may once more be dependent on the cell type and culture conditions. Since the effects of metformin on hepatic glucose production have been well documented and work in parallel to its inhibitory effects on gluconeogenesis [45], studies conducted in vivo on liver, or at least on isolated hepatocytes, may ultimately be the most informative means of investigating the cellular action of metformin. These data demonstrate that assuming a link between the change of the AMP:ATP ratio and the activation of AMPK may not always be correct, and that the change in cellular energy stores, and the activity of the kinase may, to some degree, be two phenomena that act in parallel, but not necessarily be directly related.

In a wave of studies that appeared earlier this year, the importance of AMPK in exerting the positive, health-beneficial effects of metformin has been challenged. It has been generally assumed that AMPK activation by metformin ultimately leads to the inhibition of mTORC1 [46] in a TSC1/2-dependent manner [47], or alternatively through phosphorylation of Raptor [48]. However, recent experiments performed using mouse embryonic fibroblasts from gene targeted animals demonstrated that the characteristic inhibition of mTORC1 by biguanide-derived drugs (metformin and phenformin) can occur in a manner independent of TSC1/2, and more surprisingly and unexpectedly, independent of AMPK. This surprising finding suggests that biguanides may use alternative targets to confer their beneficial metabolic/growth effects in cells by blocking TOR kinase activity, potentially through their inhibitory effects on Rag GTPases, although the effect of a true genetic Rag GTPase loss of function on the downstream effects of the biguanide drugs has not been rigorously investigated [49].

In a very important and influential study, LKB1 was shown to mediate the inhibitory effects of metformin on gluconeogenesis in the mouse liver [50]. However a recent genetic study using hepatocytes lacking functional AMPK or LKB1 showed that neither AMPK, nor LKB1 is essential for metformin-mediated inhibition of hepatic glucose production [41]. Moreover, although it is accepted that AMPK inhibits the expression of the G6Pase in rat hepatoma cells, the inhibition of G6Pase by metformin is independent of complex I and of AMPK activation [51]. Since gluconeogenesis is highly energy-consuming it is probable that the reduced mitochondrial output conferred by metformin treatment could directly impact the process and shunt any requirement for either of LKB1 or AMPK. These recent findings prompt a re-evaluation of the current, accepted paradigm of AMPK function as an obligate downstream effector of metformin action.

The last decade has seen extraordinary advances in our understanding of how diabetes, obesity, and cancer are linked to a complex cascade of protein kinase activity that impinge on AMPK, resulting in both acute and chronic effects on cellular metabolism through phosphorylation of key protein substrates. The upcoming decade will hopefully allow us to further identify and characterize these critical targets to design novel strategies around their control to ameliorate these three disorders, and perhaps many others. The examples that we have highlighted in this review underscore the importance of the use of multiple cell and animal models, since the generalized paradigms describing AMPK function have not proven robust over time when rigorously challenged. The recent new developments described in this review emphasize the critical role of genetic analysis in permitting non-biased approaches to dissecting out LKB1/AMPK function. However, these strategies will be most valuable by generating testable models that will ultimately rely on a combination of approaches ranging from complex cell biological analysis to metabolomic studies in trying to establish

the relationships between these effectors and the potential compounds that may influence their function.

Acknowledgments

We would like to thank Dr. Russell Jones for critical input and reading of the manuscript, along with an anonymous reviewer for suggestions. Work in the Roy Laboratory is supported by Operating grants from the Canadian Institutes of Health Research (CIHR).

References

[1] Hardie, D.G. and Pan, D.A. (2002) Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. Biochem. Soc. Trans. 30, 1064–1070.
[2] Narbonne, P. and Roy, R. (2006) Inhibition of germline proliferation during *C. elegans* dauer development requires PTEN, LKB1 and AMPK signalling. Development 133, 611–619.
[3] Donsmark, M., Langfort, J., Holm, C., Ploug, T. and Galbo, H. (2004) Contractions induce phosphorylation of the AMPK site Ser565 in hormone-sensitive lipase in muscle. Biochem. Biophys. Res. Commun. 316, 867–871.
[4] Narbonne, P. and Roy, R. (2009) *Caenorhabditis elegans* dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. Nature 457, 210–214.
[5] Treebak, J.T. et al. (2010) Identification of a novel phosphorylation site on TBC1D4 regulated by AMP-activated protein kinase in skeletal muscle. Am. J. Physiol. Cell Physiol. 298, C377–C385.
[6] Pehmoller, C., Treebak, J.T., Birk, J.B., Chen, S., Mackintosh, C., Hardie, D.G., Richter, E.A. and Wojtaszewski, J.F. (2009) Genetic disruption of AMPK signaling abolishes both contraction- and insulin-stimulated TBC1D1 phosphorylation and 14-3-3 binding in mouse skeletal muscle. Am. J. Physiol. Endocrinol. Metab. 297, E665–E675.
[7] Taylor, E.B. et al. (2008) Discovery of TBC1D1 as an insulin-, AICAR-, and contraction-stimulated signaling nexus in mouse skeletal muscle. J. Biol. Chem. 283, 9787–9796.
[8] Barnes, K. et al. (2002) Activation of GLUT1 by metabolic and osmotic stress: potential involvement of AMP-activated protein kinase (AMPK). J. Cell Sci. 115, 2433–2442.
[9] Marsin, A.S. et al. (2000) Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. Curr. Biol. 10, 1247–1255.
[10] Buzzai, M., Jones, R.G., Amaravadi, R.K., Lum, J.J., DeBerardinis, R.J., Zhao, F., Viollet, B. and Thompson, C.B. (2007) Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res. 67, 6745–6752.
[11] Dagher, Z., Ruderman, N., Tornheim, K. and Ido, Y. (1999) The effect of AMP-activated protein kinase and its activator AICAR on the metabolism of human umbilical vein endothelial cells. Biochem. Biophys. Res. Commun. 265, 112–115.
[12] Krawczyk, C.M. et al. (2010) Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. Blood 115, 4742–4749.
[13] Vincent, M.F., Bontemps, F. and Van den Berghe, G. (1992) Inhibition of glycolysis by 5-amino-4-imidazolecarboxamide riboside in isolated rat hepatocytes. Biochem. J. 281 (Pt 1), 267–272.
[14] Fogarty, S. and Hardie, D.G. (2010) Development of protein kinase activators: AMPK as a target in metabolic disorders and cancer. Biochim. Biophys. Acta 1804, 581–591.
[15] He, L., Sabet, A., Djedjos, S., Miller, R., Sun, X., Hussain, M.A., Radovick, S. and Wondisford, F.E. (2009) Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. Cell 137, 635–646.
[16] Koo, S.H. et al. (2005) The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature 437, 1109–1111.
[17] Kim, Y.D. et al. (2008) Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase-dependent regulation of the orphan nuclear receptor SHP. Diabetes 57, 306–314.
[18] Lee, J.M. et al. (2010) AMPK-dependent repression of hepatic gluconeogenesis via disruption of CREB.CRTC2 complex by orphan nuclear receptor small heterodimer partner. J. Biol. Chem. 285, 32182–32191.
[19] Lochhead, P.A., Salt, I.P., Walker, K.S., Hardie, D.G. and Sutherland, C. (2000) 5-Aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. Diabetes 49, 896–903.
[20] Kawaguchi, T., Osatomi, K., Yamashita, H., Kabashima, T. and Uyeda, K. (2002) Mechanism for fatty acid "sparing" effect on glucose-induced transcription: regulation of carbohydrate-responsive element-binding protein by AMP-activated protein kinase. J. Biol. Chem. 277, 3829–3835.
[21] Dentin, R. et al. (2004) Hepatic glucokinase is required for the synergistic action of ChREBP and SREBP-1c on glycolytic and lipogenic gene expression. J. Biol. Chem. 279, 20314–20326.
[22] Zhou, G. et al. (2001) Role of AMP-activated protein kinase in mechanism of metformin action. J. Clin. Invest. 108, 1167–1174.
[23] Horton, J.D., Goldstein, J.L. and Brown, M.S. (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Invest. 109, 1125–1131.
[24] McGee, S.L. and Hargreaves, M. (2008) AMPK and transcriptional regulation. Front. Biosci. 13, 3022–3033.
[25] Bungard, D. et al. (2010) Signaling kinase AMPK activates stress-promoted transcription via histone H2B phosphorylation. Science 329, 1201–1205.
[26] Sauve, A.A., Wolberger, C., Schramm, V.L. and Boeke, J.D. (2006) The biochemistry of sirtuins. Annu. Rev. Biochem. 75, 435–465.
[27] Cantó, C., Jiang, L.Q., Deshmukh, A.S., Mataki, C., Coste, A., Lagouge, M., Zierath, J.R. and Auwerx, J. (2010) Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. Cell Metab. 11, 213–219.
[28] Canto, C. et al. (2009) AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 458, 1056–1060.
[29] Donmez, G. and Guarente, L. (2010) Aging and disease: connections to sirtuins. Aging Cell 9, 285–290.
[30] Chung, S., Yao, H., Caito, S., Hwang, J.W., Arunachalam, G. and Rahman, I. (2010) Regulation of SIRT1 in cellular functions: role of polyphenols. Arch. Biochem. Biophys. 501, 79–90.
[31] Fulco, M., Cen, Y., Zhao, P., Hoffman, E.P., McBurney, M.W., Sauve, A.A. and Sartorelli, V. (2008) Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. Dev. Cell 14, 661–673.
[32] Canto, C., Jiang, L.Q., Deshmukh, A.S., Mataki, C., Coste, A., Lagouge, M., Zierath, J.R. and Auwerx, J. (2010) Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. Cell Metab. 11, 213–219.
[33] Jorgensen, S.B., Treebak, J.T., Viollet, B., Schjerling, P., Vaulont, S., Wojtaszewski, J.F. and Richter, E.A. (2007) Role of AMPKalpha2 in basal, training-, and AICAR-induced GLUT4, hexokinase II, and mitochondrial protein expression in mouse muscle. Am. J. Physiol. Endocrinol. Metab. 292, E331–E339.
[34] Zong, H., Ren, J.M., Young, L.H., Pypaert, M., Mu, J., Birnbaum, M.J. and Shulman, G.I. (2002) AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. Proc. Natl. Acad. Sci. USA 99, 15983–15987.
[35] Garcia-Roves, P.M., Osler, M.E., Holmstrom, M.H. and Zierath, J.R. (2008) Gain-of-function R225Q mutation in AMP-activated protein kinase gamma3 subunit increases mitochondrial biogenesis in glycolytic skeletal muscle. J. Biol. Chem. 283, 35724–35734.
[36] Canto, C. and Auwerx, J. (2009) PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr. Opin. Lipidol. 20, 98–105.
[37] Jager, S., Handschin, C., St-Pierre, J. and Spiegelman, B.M. (2007) AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc. Natl. Acad. Sci. USA 104, 12017–12022.
[38] Fryer, L.G., Parbu-Patel, A. and Carling, D. (2002) The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J. Biol. Chem. 277, 25226–25232.
[39] Hawley, S.A., Gadalla, A.E., Olsen, G.S. and Hardie, D.G. (2002) The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. Diabetes 51, 2420–2425.
[40] El-Mir, M.Y., Nogueira, V., Fontaine, E., Averet, N., Rigoulet, M. and Leverve, X. (2000) Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J. Biol. Chem. 275, 223–228.
[41] Foretz, M. et al. (2010) Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. J. Clin. Invest. 120, 2355–2369.
[42] Zhang, L., He, H. and Balschi, J.A. (2007) Metformin and phenformin activate AMP-activated protein kinase in the heart by increasing cytosolic AMP concentration. Am. J. Physiol. Heart. Circ. Physiol. 293, H457–H466.
[43] Hawley, S.A. et al. (2010) Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. Cell Metab. 11, 554–565.
[44] Hardie, D.G. and Hawley, S.A. (2001) AMP-activated protein kinase: the energy charge hypothesis revisited. Bioessays 23, 1112–1119.
[45] Kirpichnikov, D., McFarlane, S.I. and Sowers, J.R. (2002) Metformin: an update. Ann. Intern. Med. 137, 25–33.
[46] Tzatsos, A. and Kandror, K.V. (2006) Nutrients suppress phosphatidylinositol 3-kinase/Akt signaling via raptor-dependent mTOR-mediated insulin receptor substrate 1 phosphorylation. Mol. Cell. Biol. 26, 63–76.
[47] Dowling, R.J., Zakikhani, M., Fantus, I.G., Pollak, M. and Sonenberg, N. (2007) Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res. 67, 10804–10812.
[48] Gwinn, D.M., Shackelford, D.B., Egan, D.F., Mihaylova, M.M., Mery, A., Vasquez, D.S., Turk, B.E. and Shaw, R.J. (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. Mol. Cell 30, 214–226.
[49] Kalender, A. et al. (2010) Metformin, independent of AMPK, inhibits mTORC1 in a rag GTPase-dependent manner. Cell Metab. 11, 390–401.
[50] Shaw, R.J., Lamia, K.A., Vasquez, D., Koo, S.H., Bardeesy, N., Depinho, R.A., Montminy, M. and Cantley, L.C. (2005) The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science 310, 1642–1646.
[51] Ota, S. et al. (2009) Metformin suppresses glucose-6-phosphatase expression by a complex I inhibition and AMPK activation-independent mechanism. Biochem. Biophys. Res. Commun. 388, 311–316.

[52] Cool, B., Zinker, B., Chiou, W., Kifle, L., Cao, N., Perham, M., Dickinson, R., Adler, A., Gagne, G., Iyengar, R., Zhao, G., Marsh, K., Kym, P., Jung, P., Camp, H.S. and Frevert, E. (2006) Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. Cell Metab. 3, 403–416.

[53] Guigas, B., Sakamoto, K., Taleux, N., Reyna, S.M., Musi, N., Viollet, B. and Hue, L. (2009) Beyond AICA riboside: in search of new specific AMP-activated protein kinase activators. IUBMB Life. 61, 18–26.

[54] Pang, T., Zhang, Z.S., Gu, M., Qiu, B.Y., Yu, L.F., Cao, P.R., Shao, W., Su, M.B., Li, J.Y., Nan, F.J. and Li, J. (2008) Small molecule antagonizes autoinhibition and activates AMP-activated protein kinase in cells. J. Biol. Chem. 283, 16051–16060.

[55] Saha, A.K., Avilucea, P.R., Ye, J.M., Assifi, M.M., Kraegen, E.W. and Ruderman, N.B. (2004) Pioglitazone treatment activates AMP-activated protein kinase in rat liver and adipose tissue in vivo. Biochem. Biophys. Res. Commun. 314, 580–585.

[56] Cheng, Z., Chen, A.F., Wu, F., Sheng, L., Zhang, H.K., Gu, M., Li, Y.Y., Zhang, L.N., Hu, L.H., Li, J.Y. and Li, J. (2010) 8,8-Dimethyldihydroberberine with improved bioavailability and oral efficacy on obese and diabetic mouse models. Bioorg. Med. Chem. 18, 5915–5924.

[57] Guigas, B., Taleux, N., Foretz, M., Detaille, D., Andreelli, F., Viollet, B. and Hue, L. (2007) AMP-activated protein kinase-independent inhibition of hepatic mitochondrial oxidative phosphorylation by AICA riboside. Biochem. J. 404, 499–507.